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Distinction in Morphology and Behavioral Role between Dorsal and Ventral Groups of Cricket Giant Interneurons

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ABSTRACT—Visualization of the gross morphology of eight giant interneurons (GIs) was accomplished in 3rd instar crickets with intracellularly injected Lucifer Yellow. The GIs ascend their axons from the terminal abdominal ganglion to the deutocerebrum of brain in two recognizable groups, dorsal and ventral GIs. Comparisons of axonal arbors of the GIs showed group-specific branching pattern in the thoracic ganglia. In adults tethered without leg contact with the substratum, intracellular injection of depolarizing current into single dorsal GIs initiated flying behavior. When the legs were in contact with the substratum, the same current injection initiated walking behavior. Either depolarizing or hyperpolarizing current injected into any one of ventral GIs had no effect on behavior.

INTRODUCTION

Wind-sensitive giant interneurons (GIs), whose somata and major dendritic processes are in the terminal abdominal ganglion (TAG) and large axons ascend from the TAG through the ventral nerve cord, have been reported in several insect species [2, 3, 4, 8, 13]. In the cricket, seven GIs (7–1, MGI, LGI, 9–2, 9–3, 10–2, and 10–3) have been identified by the morphological and physiological characteristics [8]. They have long been thought to play a role in directing wind-elicited behavior such as running escape. Their axons were believed to extend through the nerve cord into thoracic ganglia and possibly into head ganglia. Due to their long size (about 25 mm length in adults), nobody has succeeded in staining entire structure of the GIs. Furthermore, because of the difficulty to access them by an intracellular microelectrode during behavior, their behavioral function has remained unsolved.

In this paper, we describe gross morphology of each GI stained individually by intracellular injection of Lucifer Yellow, revealed in 3rd instar crickets (about 5 mm length). Furthermore, we show that the behavioral function revealed by intracellular stimulation of single GIs in tethered adult crickets, correlated well with the morphological distinction between dorsal and ventral GIs.

MATERIALS AND METHODS

Adult and 3rd instar crickets (*Gryllus bimaculatus*) bred in our laboratory were used throughout the experiments. The animals were raised on a 12–12 hr light-dark cycle at the constant temperature of 28°C.

In 3rd instar crickets, single GIs were stained intracellularly by iontophoretic injection of Lucifer Yellow. For the detailed description of the

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intracellular recording and injection techniques see Baba et al [1]. Briefly, a glass microelectrode filled with 5% (W/V) Lucifer Yellow was introduced into the TAG exposed by removing the cuticle covering the dorsal portion of the abdomen and excising the viscera. When a GI was impaled, wind puffs were delivered to the cerci from various angles in the horizontal plane to aid in identifying the GI [8, 9]. After these physiological tests, Lucifer Yellow was injected into the GI by delivering a hyperpolarizing current of 2–5 nA for 2–5 min. Then, the animal was bathed in the saline for cricket [1] and maintained at 4°C for 6–12 hr to allow Lucifer Yellow to spread over the GI. Next, the nerve cord with the whole ganglia were isolated carefully, fixed in 4% formaldehyde in a phosphate buffer (pH=4.3) for more than 30 min, dehydrated in alcohol and cleared in methylsalcylate. The stained GI was photographed in wholemount under a fluorescence microscope and then drawn immediately using a camera lucida. Some of the preparations were embedded in paraffin and sectioned at 10 μm in order to establish the location of the GI axon in the nerve cord.

To introduce the stimulating glass microelectrode into the TAG, the distal halves of the fore- and hindwings were cut off. The animal was suspended in space with the pronotum glued to a supporting rod. Behavioral responses to the intracellular stimulation of single GIs in detail, they were recorded on video tape (30 frames/sec) from both sides, using two mirrors positioned around the animal. In some cases, responses of motoneurons to intracellular stimulation of single GIs were recorded extracellularly from the nerve roots of thoracic and abdominal ganglia with a pair of tungsten bipolar hook electrodes. Action potentials of a GI elicited by the intracellular stimulation were recorded from the nerve cord between the 4th abdominal ganglion and TAG by another pair of electrodes.

RESULTS AND DISCUSSION

Whole structure of each GI was visualized by intracellular staining with Lucifer Yellow, by using 3rd instar crickets. On the basis of the morphology and response property of adult GIs, the well-known seven GIs and another GI (9–1b; [6]) were consistently recognizable structures in the nymphs. As shown in Figure 1, axons of these eight GIs ascend through the nerve cord from the TAG to the dorsal region of the deutocerebrum of brain, sending out axonal branches in each of the abdominal, thoracic and head ganglia on the way. Transverse sections across the nerve cord or ganglia showed that GIs 9–2, 9–3, 10–2, and 10–3 run through the dorsal intermedial tract and GIs 7–1, MGI, LGI, and 9–1b run through the ventral intermediate tract (Fig. 2A). Hereafter, the former should be referred to as the dorsal GIs (dGIs) and the latter as the ventral GIs (vGIs). Comparisons of axonal branch projections revealed that these two groups are morphologically distinct to each other. That is, the dGIs project axonal branches extensively both medially and laterally in the thoracic ganglia, whereas the vGIs have medial branches only, though GI 7–1 has two exceptional lateral branches in the metathoracic ganglion. Compared with the length limited staining in adults, close similarity of the axonal branches between the nymphal and adult GIs was confirmed. Distinctive difference in axonal branches between the dGIs and vGIs suggest a difference in motor role.

Functional connection between a single GI and motoneurons was examined by intracellular stimulation in adults. Activities of four nerve roots, the 5th nerve root of the mesothoracic ganglion (T2R5) innervating the midleg muscles, the 3rd nerve root of the metathoracic ganglion (T3R3) innervating the flight muscles, the 1st nerve root of the 3rd abdominal ganglion (A3R1) innervating the abdominal transverse muscles, and the 7th nerve root of the TAG (A5R7) innervating the cercal muscles, were recorded extracellularly. Injection of depolarizing current (2–4 nA, 50–700 ms) into dGIs, 10–2, 9–2, and 9–3 evoked spike discharges of in all the nerve roots on both sides (Fig. 2B). The current injection into dGI 10–3 evoked spike discharges in T2R5 and T3R3 of both sides, but not in A3R1 and A5R7 of either side. So far examined, the current injection into the vGIs neither elicited spike discharges nor changed motor activity, regardless the intensity and polarity

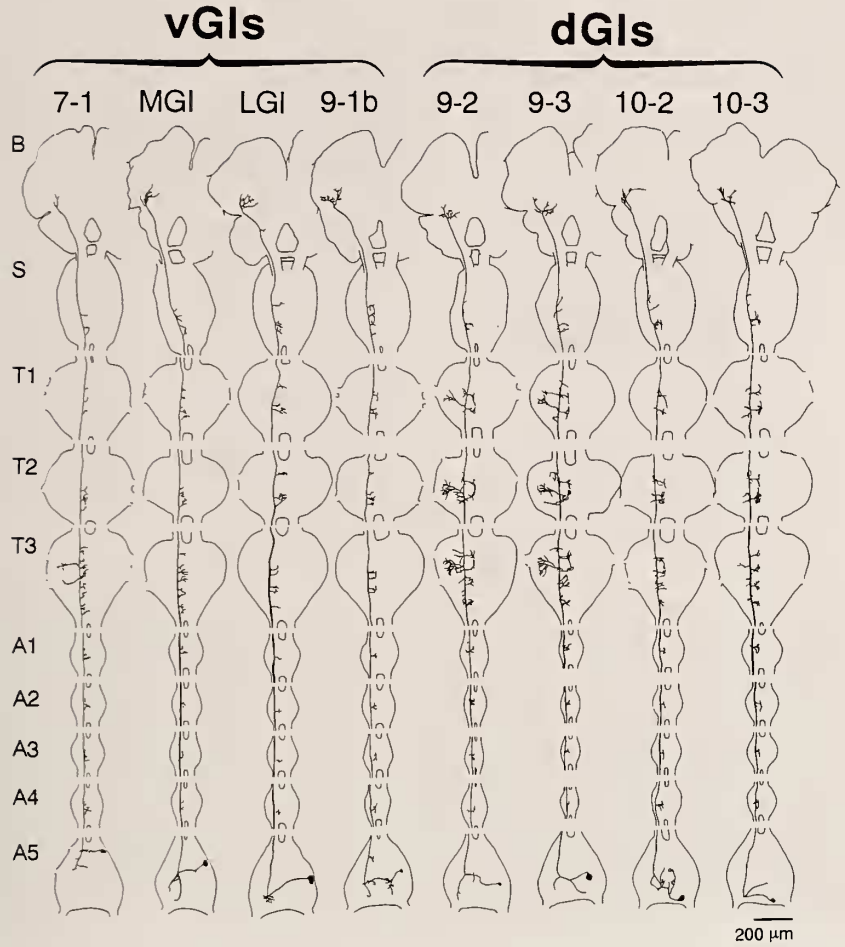

FIG. 1. Gross morphology of eight GIs in the 3rd instar nymph. (dorsal view). A1-A5, 1st to terminal abdominal ganglia; B, brain; S, suboesophageal ganglion; T1-T3; pro-, meso-, meta-thoracic ganglia; dGIs, dorsal group GIs; vGIs, ventral group GIs.

of current.

In tethered adults with the legs in contact with the substratum, injection of depolarizing current (2–4 nA) into any one of dGIs initiated walking behavior (Fig. 2Ca). The legs moved forward and backward with the tripod gait pattern, after a twitching of all legs. The current induced walking was accompanied up- and down-movements of the head and antennae (Fig. 2Ca). The stepping frequency of the induced walking was 7–8 steps/sec. This type of walking behavior is identical with the escape running in response to wind puffs on the cerci [5]. When the animals were suspended without leg contact, the injection of depolarizing current into the dGI initiated flying behavior, wing beating with legs and antennae in the flight posture (Fig. 2Cb). Minimum duration of current injection for initiating the walking behavior was 30 msec for GIs 9–2 and 9–3, and 50 msec for GIs 10–2 and 10–3. Minimum duration for initiating the flying behavior was 20 msec for GIs 9–2 and 9–3 and 30 msec for GIs 10–2 and 10–3. Walking and flying behaviors were initiated within 30 msec from the commencement of current injection, and lasted for 2–4 sec. In contrast to the dGIs, current injection into any vGI initiated no behavior at all, regardless the intensity, polarity, duration. It also had no significant effect on on-going walking or flying

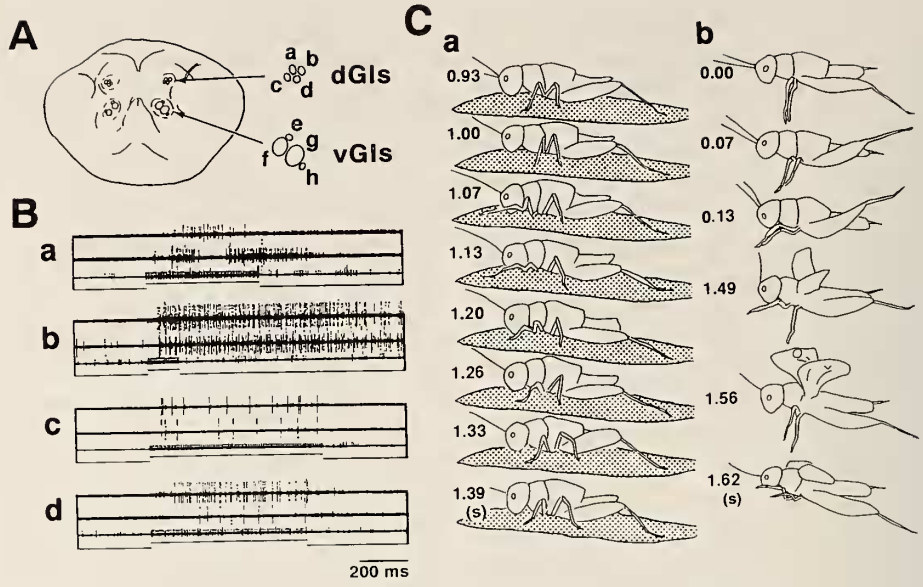

Fig. 2. (A) Semischematic illustration of dGIs and vGIs in the cross-section of metathoracic ganglion. In each hemiganglion, four large axons (a: 10–3, b: 9–3, c: 10–2, d: 9–2) ascend through the dorsal intermedial tract, and other four large axons (e: 7–1, f: MGI, g: LGI, h: 9–1b) ascend through the ventral intermedial tract. (B) Responses of motoneurons to the intracellular stimulation of dGI 9–3. In each set of records, the top is the extracellular recording from nerve root (a: 5th root of mesothoracic ganglion, b: 3rd root of metathoracic ganglion, c: 1st root of 3rd abdominal ganglion, d: 7th root of TAG) on the side of stimulated axon. The 2nd record is the extracellular recording from the contralateral nerve root. The 3rd record is the extracellular recording from abdominal nerve cord. The 4th record is the monitor of current injection. (C) Behavioral responses to the current injection into GI 9–3 (drawn from played back still pictures). a) In the animal quiescent on the substratum, the injection of depolarizing current (2 nA, 80 msec in duration) into GI 9–3 initiated the walking. b) In the same animal suspended in air, the same current injection initiated the flying. The number on the left side indicated the time in seconds after the onset of current injection.

behavior of the animal.

As described above, the dGIs differ clearly from the vGIs in morphology and functional role. The intracellular stimulation of any given dGI itself is sufficient to initiate walking or flying behavior, the behavioral pattern depending upon the legs in contact with or without substrate. Thus, it is suggested that each dGI constitutes a motor command system, and the group dGIs comprise a behavioral command system for walking and flying behaviors. In the cockroach, the dorsal and ventral groups of GIs are known as command systems for flight and running escape, respectively, though whole structure of these GIs remains unknown [10–12]. Both groups are reported to have excitatory and inhibitory influences on flight through the modulatory functions of the feedback loops between the GIs and the central flight rhythm generator in the thoracic ganglia [6].

The basic structural organization of the wind-sensitive giant interneuron system seems to be common to the cricket and the cockroach, but functional organization for the initiation of behavior may probably differ in the two species.

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